

The University of Chicago

STUDIES IN
AMPHIBIAN METAMORPHOSIS

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY
FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY

DEPARTMENT OF ZÖOLOGY

1934

By
WILLIAM ETKIN

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THE PHENOMENA OF ANURAN METAMORPHOSIS. II

(Two figures)

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E. OXYGEN CONSUMPTION DURING NORMAL METAMORPHOSIS

SINCE the classic demonstration of Gudernatch (1912) that thyroid feeding will precipitate metamorphic phenomena in the tadpole, the study of amphibian metamorphosis has furnished the field for some of the most brilliant and fruitful researches in endocrine physiology. The belief that has emerged from these researches may be briefly characterized as follows. The anterior lobe of the hypophysis produces a hormone which stimulates the thyroid gland in a way eventuating in the release of large quantities of the thyroid hormone. It is this thyroid hormone which is the primary stimulant, precipitating metamorphosis. Though this is but a crude statement, it will perhaps serve to make plain the intention of the present study.

Before much progress could be made in the further analysis of these relations it seemed to the author that a careful quantitative investigation of the nature of the metamorphic changes would have to be undertaken. The first published results dealt with the externally visible events; the rate of growth of the hind legs and body weight and the loss of water (1932).

On the basis of this study metamorphosis was divided for descriptive purposes into a prometamorphic period characterized by rapid hind-leg growth, followed by the metamorphic climax beginning

about the time of the emergence of the forelegs and during which tail resorption and modification of the mouth were the most obvious changes. This terminology will be found useful below. To this study may be added an earlier description of the development of the thyroid gland (1931). As these studies have served to clarify the nature of the changes and to give many suggestions as to their etiology, a further investigation of the changes in normal metamorphosis seemed desirable. Of the many questions that remain, one of the most interesting is the rate of metabolism of the animal undergoing metamorphosis.

It is commonly held that the thyroid secretion raises the basal metabolism of the cells, acting, as someone has expressed it, as a draft on a fire. Since, as stated above, we have every reason to believe that the thyroid hormone is the primary activator of the visible metamorphic phenomena, we would expect that some idea of the degree of activity of the thyroid in the secretion of its hormone during the various stages of metamorphosis could be estimated from the basal metabolism of the animal. The commonest measure of this metabolism being the rate of oxygen consumption in the non-working animal, the question of the respiratory metabolism during metamorphosis is at once raised.

In a previous paper I have had occasion to point out the paucity of literature on the descriptive phases of metamorphosis. The study of oxygen consumption is not exceptional in this regard. To my knowledge there is no report in the literature devoted to this subject, though a number of works touch upon it. The data of Gayda (1921a) on heat production and those of Groebbels (1922) on oxygen consumption show no effect of the inception of metamorphosis (climax phenomena) upon the metabolic rate per gram of tadpole. In both these cases, however, this point was only incidental to other purposes in the work and the individuals of the groups used were not uniform in the degree of metamorphosis that they presented, so that it is difficult to say how far this evidence can be safely interpreted. Abelin and Scheinfinkel (1923) find a drop in the total CO_2 production in one group used as control in an experimental investigation. A curve for the O_2 consumption of one individual is given by Drastich (1925). This shows a rise in early metamorphosis (metamorphic

climax) and a subsequent drop for both total and per gram respiratory rates.

It is clear that these studies are not sufficiently extensive individually or consistent collectively to permit of any conclusion. A reinvestigation of the problem covering a sufficiently large number of animals, studied under standard conditions and at known stages of metamorphosis, is plainly required.

MATERIALS AND METHODS

Specimens of the bullfrog tadpoles, *Rana catesbeiana*, obtained from an aquarium supply-house in Chicago, were used. According to the company, these animals had been collected by seining operations in Louisiana.

The animals were kept in 10 inch $\times$ 8 inch glass aquaria, four animals to the aquarium. The food consisted of algae growing in the water and also cooked, dried, and ground liver which was added abundantly every 2 or 3 days. The water was changed before feeding and the aquaria placed so as to receive several hours of sunlight per day.

The oxygen determinations were made by the Winkler titration method. The apparatus used was of the continuous flow type essentially like that described by Keys (1930), somewhat simplified. The city water supply at Chicago was used. It was first aerated in a spray device and slightly supersaturated with oxygen. The water was then led off and into the animal chambers of which twelve were arranged in a row. These chambers consisted of $7\frac{1}{2}$ -inch lengths of $1\frac{3}{8}$ -inch glass tubing. The water flowed through these and out at the opposite ends to run into the sample bottles. Two of the chambers were reserved as blanks, and into each of the other ten an animal could be introduced. It is clear that as the water flows through these chambers the animals remove a certain amount of oxygen from it. By the Winkler titration method the oxygen content of the water coming through the empty control tubes and that through each animal chamber is measured, and from this and the rate of flow the rate of O_2 consumption for the animal could be determined. The determinations were therefore made for ten individuals at a time, one such series being run each evening.

It was important to regulate accurately the rate of flow, for that determined the oxygen tension of the water in which the tadpoles were living. This was done by drawing out capillary tubing to the proper fineness, introducing these at the entrance to the sample bottles, and also by regulating the height of the aerating tank above the water bath. In this way the oxygen tension of the water from the animal chambers was kept at about 30-40 per cent saturation. In this range it has been shown for many vertebrates and for tadpoles in particular by Helff and Stubblefield (1931) that the rate of oxygen consumption is independent of the oxygen tension. In a number of cases, however, the regulation of the rate of flow was not adequate and the oxygen tension of the water leaving the animal chambers fell well below 30 per cent saturation. In such cases it was often found that the oxygen consumption fell markedly often to less than one-half previous values. Such results had, of course, to be eliminated in considering the rates at different stages of metamorphosis; but it is of some interest to notice that these data confirm in a general way the results of Helff mentioned above. It is thus clear, however, that by maintaining a saturation of about 40 per cent complications due to differences in oxygen tension are avoided.

In previous work I found that there is a marked drop in metabolism from day to night. This appeared to be correlated with the reduced activity of the animals in the dark. To take advantage of this all determinations were made at night and at approximately the same hours. The animal chambers were further screened off from the room lights. Under such conditions of reduced illumination it was found that the animals lay almost perfectly quiet for long periods of time. I have repeatedly watched the set-up of ten animals for 10-15 minutes at a time and in all have seen only a few isolated tail movements. It is clear, then, that the activity of the animal is not an important factor in the results and we are dealing here with a rate of metabolism which approximates the so-called "basal metabolism" as closely as is generally available.

Preliminary work had also shown that about an hour is required after the introduction of the animal into the chamber before an equilibrium is reached between the oxygen tension of the input and outflow waters. This is due to the fact that there are about 100 cc.

of water in the tube which must be reduced in its oxygen tension to the equilibrium point. In all experiments, therefore, the animals were permitted to remain quiet and undisturbed in the tube for 3-4 hours before a reading was taken. No attempt was made to starve the animals before introduction as it was found that 24 hours' starvation did not have much effect on the intestinal contents and interfered with the growth of the animal.

Each of the four animals of an aquarium were separately identified by small cuts in the tail fin so that individual records could be kept. In general, determinations of oxygen consumption were made every 3-4 days during the pre- and prometamorphic period and daily reading during earlier climax. The readings could be continued only until the time when the tail was one-half to two-thirds resorbed. Until this time the animals are primarily water-breathers gulping air into the lungs only occasionally, and are capable of living without access to air. However, after a definite period (when the tail is about one-half resorbed) the animals change their mode of respiration so that they will breathe only air and will not pump water through the gill chamber. At this time it is impossible to use the present apparatus, for the animals drown in the tubes, and the readings were discontinued at this point. However, for answering the questions raised, I believe the period tested to be adequate since it covers the period from before metamorphosis until the time the climax phenomena are more than one-half complete. Any effects of the factors inducing metamorphosis should therefore be apparent.

Record was taken of the body weight and of hind-leg length at weekly intervals in earlier stages and daily body-weight records were made during the climax.

In all, forty animals were investigated. For a large number, however, the records are not complete enough to permit their being used in connection with the present discussion. This is partly due to the limitation of time, partly to the loss of animals by accident during the long series of readings. As a result, the records of only twenty-four animals are sufficiently complete to cover either of the two critical stages discussed below. It will be seen, however, in the discussion of the results that the principal points can be sufficiently established from these records.

Before the results in relation to metamorphosis can be discussed it is necessary to consider the accuracy of the technique. As mentioned above, in each set-up, two control samples were taken. These agreed very well. If the two samples are averaged and the deviation of the samples from the average expressed as per cent of the average, then this population of deviations showed a mean of 0.3 per cent and a S.D. of 0.26 per cent. The repeatability of the technique is, therefore, more than adequate for the present purpose. The fact that there is a continuous flow of water through the chambers eliminates the criticism that accumulation of organic wastes interferes with the titration. Three tests for the titration of I_2 introduced into the water from the animal chamber as compared with aliquot amounts introduced into distilled water showed practically no loss. It would seem, therefore, that the Winkler titration method may be used satisfactorily in this procedure.¹

A point of considerable interest is the variation of the readings from hour to hour as the animals lay in the chamber. This was determined by making a second determination $1\frac{1}{2}$ hours after the usual one in forty-nine cases. The two determinations were then treated as the duplicate blank samples mentioned above. This population of deviations showed a mean of 4.0 per cent and a S.D. of 4.2 per cent. We have in this, then, a measure of how accurately our reading at a given time represents the true average reading for that period.

RESULTS

The numerical results for the twenty-four specimens which are suitable for our discussion are summarized in Table I for oxygen consumption and Table II for body weight.

The main tendencies of these results can best be brought out by combining the individual records. This was done by plotting the individual curves upon semilogarithmic graph paper. In this way a graph is constructed in which the slope of the line indicates the proportionate change independently of the absolute values involved.

¹ Recent work in this laboratory under Dr. Allee's direction has revealed in certain waters, and under conditions approaching those used in these experiments, a considerable error in the Winkler titration resulting from the presence of nitrites. An analysis of the water as used in the present investigation was kindly made by Dr. Schuett; this showed no appreciable amounts of nitrites.

TABLE I
SHOWING THE OXYGEN CONSUMPTION OF TADPOLES

DATE	SPECIMEN NUMBER SERIES 2BR																			
	2	3	4	6	7	8	11	12	13	15	16	18	20	23	25	26	33	34	35	36
May 20																				
21						2,096			2,733			3,125								
22	2,021	3,001									0,972									
23														1,588		0,773	2,053	1,861	1,078	
24																				
25																				1,082
26																				2,049
27																				
28																				
29																				
30																				
31	3,042*	2,752	2,715	2,966						1,760	0,832									
June 1																				
2														1,192		2,220	1,760	1,620	1,730	1,750
3		2,724	3,210																	
4																				
5	2,153																			
6	1,831	2,767	2,828							2,141	2,159			1,753		1,503	1,700	1,574	1,568	2,108
7	1,899																			1,583
8																				1,002
9																				
10																				
11																				
12																				
13																				
14																				
15																				
16																				
17																				
18																				
19																				
20																				
21																				
22																				
23																				

The day the hind legs reached to mm. in length is indicated by † and the day on which the forelegs emerged is indicated by *. The figures are given in terms of cubic centimeters of oxygen (at 20° C. and 760 mm. pressure) consumed per animal per minute. They have, however, been multiplied by 10⁶ in order to eliminate the decimal point.

TABLE I—Continued

SPECIMFN NUMBER SERIES 2BR																									
DATE	2	3	4	6	7	8	11	12	13	15	16	18	20	23	25	26	33	34	35	36	37	38	40	42	
June 24																									
25								3.625*		3.502	1.886	2.549	3.240	2.250	2.516	1.101									
26								3.420				2.071	2.420	2.420*			2.600	2.648	2.506	1.772		2.013	2.414		
27								3.010			2.258†	2.504	2.087		2.287									1.301	
28								2.520			2.524			2.286	1.090	3.078	3.332	2.021	2.456	2.572					
								2.680			2.422			2.162	2.140	1.112	2.851	2.312	2.622	1.790	2.234				
29								3.145			2.700				2.143										
								3.730			2.497				2.020										
30								2.646			1.864			3.062	2.531	2.210	1.512	2.566	2.789						
July 1								2.570							2.230										
2								2.348			2.945			3.368	2.706		1.570	3.427	3.122	3.056	2.534	2.673	2.584	2.925	2.525†
3																									
4																									
5											2.233			3.293	2.321		1.533	3.324	3.091		2.314	2.812	3.010	2.707	
6																									
7														3.020	2.682		1.101	3.304	2.862		2.041	1.807	2.323	2.390	
8											1.650			3.144			1.412	2.802	2.908			2.356			2.553
9																									
10														2.784*	2.789			3.324	2.867		2.619	2.593	2.486	2.776	2.694
11																									
12											2.848										2.574	2.683	2.550		2.828
13								2.752					2.700	2.054		1.450	2.773	2.152							
											2.570		2.915	2.923		1.421	3.538	2.185*							
14													2.404				3.470	2.381							
													2.407				3.581	3.017							
15																	1.574	3.114*	2.106		2.301	2.368*	2.427	2.457	
16											2.486										2.905				
17																					2.131				
18																					2.552		2.015		2.501
19																					2.906		2.097		2.246*
20																					3.257				2.613
21																					2.557				2.030
22																					2.200				2.130
																									1.788
																					2.335				

TABLE II
BODY WEIGHTS OF METAMORPHOSING TADPOLES

DATE	ANIMAL NUMBER																							
	2	3	4	6	7	8	11	12	13	15	16	18	20	23	25	26	33	34	35	36	37	38	40	42
	Body Weight in Grams																							
May 22.....	11.8	11.1	13.0	13.0	11.6	12.5	9.7	10.2	10.6	8.0	6.4	10.0	7.4	5.5	9.9	3.0	9.1	8.7	5.6	6.6	6.6	9.2	7.8	9.4
June 1.....	10.3	11.2	13.4	12.6	10.6	12.8	11.8	12.0	12.8	8.2	6.8	12.2	6.4	6.0	10.6	3.0	9.4	9.6	5.6	6.9	8.1	7.5	8.8	8.8
5.....	8.2	10.1	13.3	12.9	10.9	13.1	10.2	10.7	11.2	7.6	6.2	11.2	6.1	5.6	9.6	...	9.2	8.8	6.4	7.0	8.6	7.7	8.9	...
8.....	7.0	9.7	11.6	12.3	10.9	12.8	11.1	11.4	11.9	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
12.....	6.5	10.5	10.4	12.5	9.5	12.1	9.5	11.1	9.5	8.1	6.5	13.0	7.3	5.6	10.7	2.8	10.0	9.3	6.6	...	...	...	...	9.2
14.....	...	10.4	10.0	11.7	9.3	12.0	9.7	...	8.5	...	...	...	...	...	...	...	...	9.0	...	...	...	...	...	...
19.....	...	8.6	...	9.5	7.8	10.4	8.0	12.3	...	10.0	7.3	13.2	8.9	6.1	11.9	...	11.2	10.0	7.9	8.5	11.0	9.1	10.3	...
21.....	...	...	...	...	6.7	9.0	7.1	12.1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
26.....	...	...	...	...	5.2	6.2	5.5	10.6	...	10.9	6.6	9.8	10.1	6.6	10.3	4.0	12.6	11.2	9.6	9.2	11.8	10.0	11.4	...
27.....	...	...	...	...	...	...	...	10.3	...	...	...	9.5	...	...	9.6	...	...	...	...	...	...	...	...	...
29.....	...	...	...	...	...	...	...	9.8	...	...	...	8.5	...	8.2	...	...	...	...	...	...	...	...	...	...
July 1.....	...	...	...	...	...	...	...	9.1	...	...	7.4	10.2	10.2	7.8	7.1	4.6	12.0	10.9	9.9	9.6	11.3	9.8	10.8	7.4
3.....	...	...	...	...	...	...	...	8.4	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
7.....	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
10.....	...	...	...	...	...	...	...	...	...	...	7.6	...	...	8.2	...	5.3	13.0	11.3	10.5	11.4	9.8	11.8	8.1	...
13.....	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
15.....	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
17.....	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
20.....	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
22.....	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

In this form, therefore, the graphs can be superimposed without regard to the differences in absolute values. The upper sets of points in Figures 1 and 2 show the results obtained by superimposing the points according to the following procedures.

For each graph the best straight-line fit determined by inspection was drawn. A piece of thin celluloid sheet was then placed over the graph. This was adjusted so that the X-axis was exactly parallel to a line drawn on the celluloid. The value of the curve for the day the hind leg reached 10 mm. was adjusted to be just beneath a point marked on the celluloid which we shall call the "fulcrum." The points of the graph desired were then marked on the celluloid. This was done on the same piece of celluloid for each of the thirteen specimens which covered the period satisfactorily from before prometamorphosis to the day before emergence. Figure 1 shows the resulting distribution of the points.

Another similar procedure was followed using the value of the curve at two days before emergence of the forelegs as a fulcrum and covering the points in prometamorphosis and the metamorphic climax, until the last available day, i.e., the time the tail is about one-half to two-thirds resorbed. Figure 2 shows the results of this procedure.

Each graph gives us a picture of the changes in oxygen consumption with respect to some definite period in the metamorphic process. By setting the level of the fulcrum at 100, we can, by simply setting down the proper logarithmic intervals, represent the percentage deviation of any point from this value.

The period of prometamorphosis.—From Figure 1 we can see that there is an increasing oxygen consumption as the development of the animal proceeds from considerably before the onset of prometamorphosis to the day of emergence of the forelegs. No specific change appears with the onset of prometamorphosis (i.e., H.L.L. = 10 mm.).

Since our primary interest is with the rate of metabolism of each unit of tissue, we must take the growth of the tadpole into consideration. For showing the general tendencies of the body growth in weight, the same procedure of plotting the values on semilogarithmic paper and superimposing the points seemed most advantageous. The lower parts of Figures 1 and 2 show the results, using the same

specimens and the same points in metamorphosis as fulcra as in the upper curves.

We are immediately struck by the similarity of the curves for total metabolism and for body weight. Not only are they similar in form, but the percentage deviation from the fulcrum-point level is apparently much the same. In short, as the body weight increases the total metabolism increases in direct ratio so that it is clear that

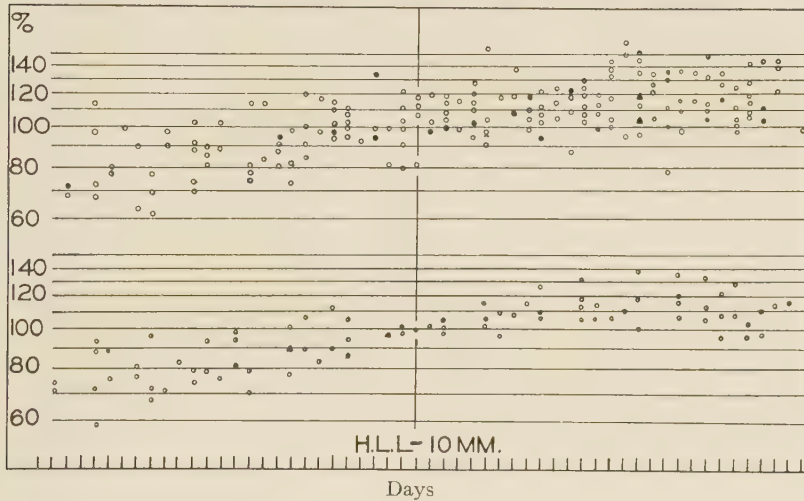


FIG. 1.—Shows the distribution of points resulting from the superposition of individual curves for thirteen animals. The day the hind legs reach 10 mm. is used as fulcrum. Upper curve for O₂ consumption and lower for body weight. Open circles indicate one reading, filled-in circles indicate two readings at the same spot, triangles indicate three readings, and a large square indicates four readings.

the rate of respiration per unit weight remains approximately constant throughout the period studied. This holds until a few days before emergence of the forelegs, when a drop in weight complicates the situation. The divergence at this time will be discussed later.

It is not satisfactory, however, to leave the analysis at this point, for I have previously shown (1932) that the growth phenomena of the tadpole at this time are complicated by a relative loss of water, so that the rate of growth of the animal in dry weight is greater than the total weight growth rate. These results were confirmed for the present batch of animals by determining the water content of the

animals left when it became necessary to discontinue the determinations. The figures obtained are shown in Table III.

If these results are compared with my previously published figures, a good agreement is readily seen. I think we are therefore justified in applying the general concept arrived at in this previous study to the present group of animals by saying that there is a gradual relative loss of water during growth previous to prometamorphosis and an acceleration of this loss during prometamorphosis. In the climax an exceedingly rapid desiccation of the tissues takes place.

TABLE III
DESICCATION PHENOMENA IN METAMORPHOSIS

An. No. Series 2BD	Description	Per Cent	Dry Weight
			Wet Weight
5.....	Early prometamorphosis	11.9	
3.....	Mid-prometamorphosis	11.3	
6.....	One day before emergence	14.9	
2.....	Mid-climax	16.8	
4.....	Mid-climax	15.6	
1.....	Complete metamorphosis	20.3	

If we apply this concept to the data given above, it will become apparent that the rate of respiration per unit dry weight decreases as the animal grows larger and the rate of decrease is accelerated during prometamorphosis. The amount by which this rate is decreased during prometamorphosis may be estimated at 30 per cent since that is approximately the difference in the amount of dry weight represented by a unit of total weight at the beginning and end of prometamorphosis.

We may summarize our consideration of this period as follows:

1. As the tadpole develops the rate of oxygen consumption rises, the rate per unit of total weight remains approximately constant, and the rate per unit of dry weight decreases.
2. In comparing pre- with prometamorphosis we find that the rate of increase of the total oxygen consumption and the rate of metabolism per unit of total weight remain unchanged and unaffected by prometamorphosis and the rate per unit of dry weight is decreased faster in prometamorphosis than previously.

Metamorphic climax.—For a similar consideration of the changes occurring at the beginning of the metamorphic climax, Figure 2 will serve. It is based upon curves for fifteen animals, only four of which are also to be found in the previous group. Here we notice that the period of prometamorphosis represented to the left of the arrow shows the same characteristics as displayed by this period in Figure 1. At about the day of emergence, however, we notice there is a

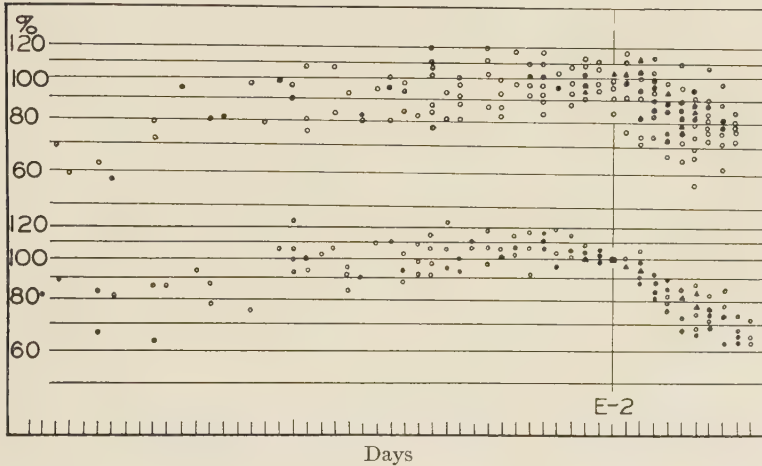


FIG. 2.—Shows the distribution of the points resulting from the superposition of individual curves for fifteen animals. The fulcrum is set at 2 days before the emergence of the forelegs. Arrangement as in Figure 1.

sharp drop in the total oxygen consumption, which continues as long as the present experiment could be run (up to 7 days after emergence). If we consider the body weight, again we are struck by a decided agreement. The drop in body weight begins somewhat before the drop in oxygen consumption, but otherwise the curves are almost identical even in the approximate percentage of the drop. It is clear, therefore, that within the variation of the figures there is a fairly constant rate of metabolism per unit body weight through prometamorphosis and early climax, with the exception that there is a slight rise in this rate as a result of the earlier falling-off of the body weight while the total oxygen consumption remains high.

Again if we consider the changes in water content, I have previ-

ously found that the period of metamorphic climax, the first one-half of which is represented in our figure, is characterized by a very rapid desiccation. It is clear, therefore, that if the rate of respiration per unit body weight remains constant, the rate per unit dry weight must fall sharply. The figures of my previous study on the rate of desiccation are not sufficiently extensive to make possible an accurate estimation of the amount of drop involved in this short time, but the fact that the tadpoles during early metamorphic climax are undergoing rapid desiccation seems perfectly clear. With regard to the slight increase coming just before the emergence of the forelegs, I am of the opinion that it has no significance in the sense of a fundamental rise in the metabolism of the animal. This appears on two grounds: (1) There is no increase in the rate of change of the total metabolism as there must be if the animal were to undergo a fundamental increase of the metabolic rate. (2) The loss of weight, which is the basis of the increased metabolic rate per unit body weight, has been shown in my previous studies to be a result partly of the loss of intestinal contents and partly to the beginning of the characteristic rapid dehydration of the metamorphic climax.

We may therefore summarize our understanding of the metabolic rate of the tadpole in relation to the early metamorphic climax as follows:

1. The rate of oxygen consumption of the animal as a whole drops more than 20 per cent during the first few days of the metamorphic climax, i.e., after emergence of the forelegs. The rate per unit of the total weight remains approximately constant, owing to the loss of body-weight coincidentally with the drop in metabolism. The rate of oxygen consumption per unit of dry weight drops markedly.

2. The rate at which the oxygen consumption per unit dry weight is decreasing is faster during early climax than previously.

Thus far our consideration of the results has been confined to the general tendencies. In a developmental process such as metamorphosis we are led to expect that the essential changes would show a high degree of constancy in the time and nature of their appearance. It is a matter of interest to know how far we can extend our conclusions to individual animals. For this purpose the available facts on

the respiratory rate and growth changes for the individual animals must be consulted. It is impractical to publish the 24 graphs available, but Table I is so arranged as to give as graphic a representation of the available data on oxygen consumption as could be obtained. When these are examined, we find that of the fifteen specimens showing a sufficient number of readings on either side of emergence (specimens 2, 3, 4, 6, 7, 8, 11, 12, 13, 18, 20, 34, 38, 42), all can be seen to show a decided drop at or soon after the day of emergence. That this drop must be real in each case can readily be seen if the relatively small variability of the successive readings on the same animal is recalled. Some variation appears in just when the drop first occurs, as in specimens 8 and 12 it is 2-3 days after emergence and in specimens 20 and 34 apparently on the day before or day of emergence of forelegs. It seems sufficiently clear, therefore, that a drop in total metabolic rate must be considered a characteristic event of early climax taking place in all normally metamorphosing animals.

The prometamorphic changes in metabolic rate, lacking as they do the striking definiteness of the change at emergence, are more difficult to follow in individual cases. The author believes, however, that an examination of Table I will reveal that in each adequate case there is a decided tendency for the results to conform to the general tendency revealed by the combined results. We are led to believe, therefore, that the oxygen consumption, like the other metamorphic events studied, displays a regular, perfectly definite change such as has been summarized above for the group as a whole.

DISCUSSION

In the introduction the expected results of a study of the metabolism of metamorphosing tadpoles was discussed. It was there pointed out that according to current theory a distinct increase in oxygen consumption was to be anticipated. The actual results found in the present study are diametrically opposed to this.

This contradiction compels us to re-examine the grounds of our reasoning. As previously stated, the direct evidence on the metabolic rates of metamorphosing amphibia is very scarce and unsatisfactory. The assumption, nevertheless, that there is a heightened

metabolism accompanying and perhaps of causal significance in the transformation of these animals is very general (thus see Jahrish, 1920; Uhlenhuth, 1921; Huxley, 1925; Schulze, 1930; Adams, Kuder, and Richards, 1932).

Uhlenhuth, for example, writes (1921, pp. 197): "From these facts it seems evident that the amphibian metamorphosis is the result of a highly increased metabolism, or, more correctly, metamorphosis seems to result if metabolism is increased in such a degree and manner that catabolism becomes higher than anabolism."

The most direct evidence brought forward for this view of metamorphosis seems to rest for the most part on several facts.

1. There is a considerable loss in weight in metamorphosis.
2. The rise in metabolism is considered the most characteristic effect of hyperthyroidism in mammals.
3. Substances presumed to increase metabolism have been reported to accelerate the metamorphosis-inducing effects of thyroid treatment and vice versa (thus Jahrish, Uhlenhuth, Huxley as above).
4. Thyroid medication is reported to accelerate the metabolic rate of larval amphibia in which metamorphosis is produced (discussion below).

A brief consideration and criticism of these points seems to me necessary for a clarification of the problem.

The loss of weight in normal metamorphosis I have previously shown (1932) to rest almost if not entirely upon the loss of intestinal contents and water. It cannot therefore be presumed a priori to be evidence for increased catabolism. The argument from the work on mammals is at best an unsafe analogy; the third point above assumes that an increase in metabolism takes place rather than proving it. The last point is, however, the most direct and seemingly the most conclusive in indicating that a rise in metabolism must at least accompany metamorphosis.

On the subject of metabolism of thyroid-medicated amphibia I am familiar with the following reports: Gayda (1921*b*) Groebbel (1922), Abelin and Scheinfinkel (1923), Helff (1926), and Belehrádek and Huxley (1927). Abelin and Scheinfinkel report an initial slight rise in total CO₂ production followed by subsequent marked fall.

The other authors mentioned have considered not the total metabolism but the metabolism per unit body weight, and have uniformly come to the conclusion that at some time in the artificial metamorphosis precipitated there is a rise in metabolism. Groebbels, Helff, and Bělehrádek and Huxley have worked on oxygen consumption while Gayda has used heat production as a measure of metabolic rate. If we use the more complete reports of Helff and Gayda and estimate from their data the total metabolism of their animals, we find that they agree with that of Abelin and Scheinfinkel in showing a decrease with the process of metamorphosis. In this respect, then, the work on thyroid medication is in essential agreement with my results on normal metamorphosis.

And yet at first sight the insistence of the more recent workers (Helff, also Bělehrádek and Huxley) upon using the rate per gram of body weight as a basis of discussion seems justified, and it is on this basis that a rise in metabolism is predicated. But a more careful consideration will, I think, throw serious doubt upon the value of this procedure. In the first place, we find that one of the most striking results of thyroid feeding is a sharp decline in body weight. From the studies of Romeis (1915) and the comparison with a similar drop in normal metamorphosis mentioned above, it is clear that a large part of the absolute weight loss is a result of dehydration and intestinal evacuation. If the conditions are comparable to normal metamorphosis (which, of course, is the assumption upon which the foregoing worked), then a unit of total weight would assume entirely different values in terms of the amount of protoplasm represented at different stages in artificial as well as in normal metamorphosis. Therefore, the use of the total weight as a measure of the amount of metabolizing tissue is seriously to be questioned. In the second place, a rising in metabolism should show up initially in the total metabolism, as indeed is the case in thyroid medication in mammals. This, however, does not appear in the reports mentioned above except that of Abelin and Scheinfinkel.

For these reasons I am of the opinion that the question of the influence of thyroid upon larval amphibia must be considered as unsettled and indeed must remain so until more is known of exactly

what the body-weight changes that result from thyroid medication involve.

It must be considered, therefore, that an accelerating effect of the thyroid treatment upon the metabolism of larval amphibia has not been demonstrated. It then becomes understandable that we should have failed in finding an increased metabolic rate in metamorphosis, for we see that the fundamental assumption upon which our expectations were based—namely, that the thyroid secretion stimulated basal metabolism—was not warranted as applied to the amphibian larva. We are therefore free to draw the natural conclusion that thyroid hormone, in quantities such as are involved in the normal induction of metamorphosis, does not raise the metabolic rate of amphibian larvae. In this connection it is of great interest to note that Henschel and Steuber have recently shown (1931) that the adult frog does not respond to thyroid treatment by any increase in metabolic rate.

The problem of the cause of the drop in metabolism in early metamorphic climax cannot of course be solved from the available data. An important contributing factor may be pointed out, however. It is well known that a cessation of feeding results in a drop in basal metabolism. This has been found for animals generally, for example, Bělehrádek and Huxley (1927) in the axolotle. Since the tadpole in the metamorphic climax ceases to feed and quickly empties its intestine, it would appear that some part of the drop, at least, is likely due to this factor. A test of this point is difficult in the tadpole, for in ordinary starvation the intestine is very slowly emptied so that conditions comparable to those of normal metamorphosis are not attained. Other possible factors contributing to a decrease such as the change in the hydration of the tissues might be mentioned but cannot as yet be evaluated.

A point of general interest in these data on the metabolic rate of the developing tadpole may be pointed out. It has been found to be a general rule that the metabolism of an animal per unit weight decreases with age. As we have seen above, this does not appear to be the case in the bullfrog tadpole over the later part of its existence as such. When, however, the data are analyzed in terms of the rate of metabolism per unit dry weight, the tadpole is seen to fall clearly in

line with other animals investigated. We see here another example of the importance of taking into account the peculiarly rapid desiccation of the tissues that the tadpole undergoes in any consideration of the growth of the animal.

SUMMARY

An investigation of the rate of oxygen consumption of bullfrog tadpoles in relation to the various stages of metamorphosis was made. The conclusions drawn may be summarized as follows:

1. During the period of rapid hind-leg growth (prometamorphosis) the total O_2 consumption rate rises, but the rate per unit tissue remains constant. The rate per unit dry weight falls more rapidly than previously.

2. During the first part of the period characterized by the involution of larval organs (metamorphic climax) the total rate falls coincidentally with the body weight. The rate of metabolism per unit dry weight falls rapidly.

A consideration of the literature further strengthens the conclusion that there is no reason at present for ascribing any causative significance in metamorphosis to the supposed metabolism-accelerating influence of the thyroid. This supposition itself is indeed not adequately supported as applied to larval or adult frogs.

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THE MECHANISMS OF ANURAN METAMORPHOSIS

I. THYROXINE CONCENTRATION AND THE METAMORPHIC PATTERN ¹

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FIVE FIGURES

INTRODUCTION

Although the essential role of the thyroid hormone in inducing anuran metamorphosis has been examined in a variety of ways (for literature, see Allen, '29) yet quantitative refinements of such experiments have been seldom attempted. According to Romeis ('23) the metamorphic changes induced by thyroxine are similar to those resulting from thyroid feeding and are a function of the concentration and the duration of exposure. Blacher ('28), in animals killed after exposure for 3 days to a suspension of desiccated thyroid, noted that certain structures such as intestine and hind legs are more sensitive than others such as the tail, and that such differences are related to the normal sequence of metamorphic events, the structures most responsive to thyroid being in general the first to show metamorphic changes. Allen ('32) immersed tadpoles in various concentrations of thyroxine and also varied the duration of exposure. He concluded that the response depends not only on concentration, but on the time during which the solution acts. The order of sensitivity of parts which he reported is similar to but not identical with

¹The greater part of this work was done at the Hull Zoölogical Laboratory, The University of Chicago, as a thesis for the doctorate under Dr. C. R. Moore to whom I am indebted for advice and encouragement. The results were checked and extended in the Laboratory of Experimental Biology, American Museum of Natural History for the courtesies of which thanks are due to Dr. G. K. Noble.

that found by Blacher. Kosmin and Resnitschenko ('27), using body length as a criterion of progress in metamorphosis in some work on the effects of different concentrations of thyroxine, added age as a factor in the result, older tadpoles responding more markedly. Clausen ('30) found muscle and skin from the anterior part of the tail more responsive to the metamorphosis inducing factor than similar tissues from posterior tail regions.

In a previous study (Etkin, '32) I have described the definite sequence of metamorphic events as observed in the living animal. It was shown that these events not only follow in a set order, but that the changes are so spaced as to allow adequate time for the expression of each. For example, the hind legs enjoy a long period of rapid growth before tail resorption has progressed so far as to render the tail useless as a locomotor mechanism. Many similar adaptive relationships are noticeable in the metamorphic time-table. Such findings sharply present the problem: how are the agents inducing metamorphosis able to correlate the individual changes into a harmonious whole? The question is evidently one of embryonic correlation, and must be investigated from that standpoint. Fruitful researches in this direction have already been made, chiefly by Helff and his students (for summary and references see Helff, '31), and they have uncovered one of the mechanisms involved. They have shown that some metamorphic events are not direct responses to hormones but are induced by neighboring structures, as is the case with the formation of the tympanic membrane, the perforation for the emergence of the fore legs, etc. It appears, however, from their work that the correlating factors of induction do not explain all of the metamorphic inter-relationships, many of which seem to be hormonally controlled. My study (Etkin, '32) of the relation of the development of the thyroid gland to metamorphosis suggests a possible mechanism for the spacing of metamorphic events, for that study indicated that the gland becomes increasingly active during early metamorphosis. Consequently, if different organs and parts have intrinsically different thresholds of

response to the thyroid hormone, a spacing of those metamorphic events dependent on thyroxine concentration would result.

Close study and observation of the effect on metamorphic changes of thyroxine administration in graded quantities should offer a method of testing the validity of this suggestion. The work of Blacher and Allen cited above already indicates that different organs and parts of the tadpole do respond to different concentrations of thyroid hormone, with duration of exposure as a complicating factor. A more extensive investigation, however, seems desirable and is offered in the present paper.

MATERIALS, METHODS, AND PRELIMINARY DATA

The species used was the western wood-frog, *Rana cantibriensis*, sometimes included as a variety of *Rana sylvatica*, which it very closely resembles. The animals came from two clutches of eggs (designated clutches 1 and 7), collected near Gary, Indiana. Several hundred tadpoles of each clutch were operated upon for the removal of the thyroid anlage by a technique much the same as that recommended by Allen ('29). The percentage of successful operations was determined by allowing a group of tadpoles to pass through the metamorphic period. Those animals which failed to show any signs of metamorphosis (rapid hind leg growth) 3 weeks after the vast majority of controls and unsuccessfully operated animals in the same tanks had metamorphosed, were considered to have been operated successfully. Of about sixty animals of each of the two clutches kept under observation for this purpose, 25 per cent of batch 1 and 64 per cent of batch 7 appeared to have been adequately thyroidectomized. This percentage is considerably below that of other workers, Allen for instance reporting over 90 per cent of successful operations, and is probably largely the result of my inexperience with the technique. As concerns the sequel, we need only bear in mind that when a considerable number of young operated tadpoles are used in an experiment some will have been thyroidectomized and others not.

The experiments consisted in subjecting individual operated animals to various concentrations of thyroxine and observing the effects in the living state. Synthetic crystalline thyroxine (Hoffman-LaRoche & Co.) was used. A stock solution was made up with quantitative accuracy by dissolving about 0.01 gm. of thyroxine in 5 cc. of 0.1 per cent NaOH and then diluting with tap or distilled water to give a concentration of 1 part thyroxine in 100,000 parts of water. Other concentrations were prepared by diluting this solution. For convenience the concentrations used will be designated by the exponent of 10 needed to express the number of parts of water per part thyroxine; thus 7 means 1 part thyroxine in $10,000,000 = 10^7$ parts of water, $7\frac{1}{3}$, 1 part thyroxine in $21,500,000 = 10^{7\frac{1}{3}}$ parts water, etc. Control animals in water not containing thyroxine are indicated by the symbol α .

Concentration 5 was never used more than 3 days as by that time it generally formed precipitates. Concentration 6 was usually employed as a basis for dilution and was generally made up fresh every 2 weeks or so, remaining clear during such periods. Only for concentration 5 was it found necessary to adjust the pH to that of the water used for dilution. The animals not being used in experiments were kept in aquaria with algae and generally also a piece of liver. Experimental animals were placed singly in finger bowls in about 100 cc. of thyroxine solution, and were fed for 1 to 2 hours daily on cooked, ground liver and ground algae added to their dishes or put into other dishes to which the animals were temporarily removed. In the former case, the solution had to be changed after the feeding period. In the latter case, the solution was changed every 3 to 5 days.

The metamorphic time-table derived from previous studies (Etkin, '32) has furnished the basis for the selection of the characters studied in the present experiments and must be kept in mind throughout for the understanding of procedures and results. Since, however, that work concerned other species of *Rana*, it has been deemed necessary to obtain similar values for the species here employed. For this purpose

ten normal animals were observed through the later tadpole and the metamorphic period and dry weight determinations covering the same periods were also made. The results accord with those reported for other species. Figures 1 and 4 give examples of the sequence of metamorphic events. The early metamorphic period termed prometamorphosis is characterized externally by the rapid growth of the hind legs which in this species (as in small species generally) continues through the metamorphic climax. Late in prometamorphosis the anal canal piece, a lobe of tissue through which the posterior end of the gut is extended along the ventral tail fin for a short distance, is absorbed. On entrance into the climax period, an area of skin degeneration, termed skin window for the fore legs (S.W.F.L.) appears in the operculum. On the left side the spiracular tube is resorbed. In the mouth a sequence of events occurs beginning with the reduction of the lips and a loss of their horny teeth, followed by the shedding of the horny beaks, and a gradual widening of the mouth to its full frog character. Reduction of the tail begins simultaneously with the resorption of the lips and progresses synchronously with the widening of the mouth so that both tail resorption and mouth development are completed at about the same time. The time relationships of these events are graphed in figures 1 and 4. Although only those changes discernible externally without injury to the animal have been recorded, yet these form a sequence spread throughout the course of metamorphosis. The method is therefore adequate for a study of metamorphosis and further allows continuous observation of the same animal and accurate timing of the various events.

In attempting to analyze the effect of various thyroxine concentrations on the metamorphic sequence, I felt that close and continuous observation of individual living animals is essential. This is the most important respect in which the procedure here employed differs from that of Blacher and Allen, who relied on comparisons of populations killed at definite times. Although more laborious, the method of individual examination has fully justified itself by the wide range

of usable information and richness of detail yielded. Complete records of all changes observed and pertinent measurements especially of hind leg length were kept. Limb buds were measured with a micrometer eyepiece, larger limbs with vernier calipers. Where the rapidity of change warranted, observations were made morning and evening (experiment 3 only), otherwise daily or less frequently.

Great difficulty was experienced in attempting to raise these tadpoles through metamorphosis, even unoperated ones frequently succumbing. Frequent handling is probably the chief cause of this poor viability although other factors doubtless contribute. It has therefore been possible to carry the experimental animals only well into the last half of the metamorphic climax. The final events as completion of tail resorption and widening of the mouth could not be studied. However, death in the late stages of metamorphosis does not detract from the conclusions since all of the essential events studied were far advanced by that time.

Since the conditions of experiment 3 were best controlled, examples from this experiment will be most often used for illustration. Where this is done, it should, however, be understood that the data of the other experiments completely accord with the statements made.

EXPERIMENTS AND RESULTS WITH GRADED THYROXINE CONCENTRATIONS

1. Details of procedure

Tests of the effect of exposure to various concentrations of thyroxine comprise three lots of experiments, which will hereafter be designated as 1, 2 and 3.

Experiment 1. The animals, coming from clutch 1, were less than half grown at the start of the experiment, measuring 16 to 24 mm. total length and weighing about 0.1 gm. (Metamorphosis began in the unoperated controls at a length of about 40 mm. and a weight of about 0.5 gm.) Ten operated animals were placed singly in each of the following concentrations of thyroxine: 6, 7, 8, 9, 10; and thirteen operated animals were kept in tap water as controls. The dry

weights were determined for one animal of each group before the experiment proper began and at convenient occasions thereafter.

Experiment 2. Animals from clutch 7 and of about the same size as those in experiment 1 were used. Five operated animals were placed singly in each of the following concentrations: 6, $6\frac{1}{3}$, $6\frac{2}{3}$, 7, $7\frac{1}{3}$, $7\frac{2}{3}$, 8, $8\frac{1}{3}$, $8\frac{2}{3}$ and 9. In addition, groups of unoperated control animals from clutches 7 and 3 were included at concentration $7\frac{1}{3}$.

Experiment 3. Clutch 1 animals were employed, mostly three-fourths grown (length, 30 to 35 mm., weight 0.3 to 0.4 gm.); but each group included one nearly full grown tadpole. The same concentrations were used as in experiment 2 with the addition of $9\frac{1}{3}$ and $9\frac{2}{3}$, four operated animals being subjected to each concentration. Two extra groups of animals as large as or larger than the size at normal metamorphosis were selected from a tank which had received special care to induce rapid growth and included at concentrations $7\frac{1}{3}$ and 8. The temperature was controlled at $26\pm 2^{\circ}\text{C}$.

In all experiments the animals were kept alive in the solutions as long as possible and consequently the last observation recorded was generally followed by death. They were preserved in Bouin's fluid and where the state of preservation warranted, serial sections of the head back to the heart were made for study of the results of the thyroidectomy.

2. Behavior of controls

In no case did any of the operated controls kept in tap water show any metamorphic changes. This was to be expected in experiments 1 and 2 for the growth attained under the conditions of the experiment was insufficient to bring on metamorphosis even had the animals possessed thyroids. In experiment 3, normal metamorphosis might have been expected but failed to appear in any of the four controls. On histological examination no thyroid was found in two of these, only a small thyroid in one, while the fourth animal died before fixation. Since hind limb buds lengthen with body

growth before metamorphosis, the curves of hind limb length will show slight increases even in controls which never metamorphose. Therefore in the present experiments, the effect of a given solution can be judged only when hind leg growth is much greater than in the controls.

3. Comparison of results in the three experiments

The results of the three sets of tests agree so closely that they may be considered together. Although relatively few animals were subjected to any given concentration, the high uniformity and consistency as regards fundamental points yield clear-cut interpretations and justify the use of individual examples.

In general, as was to be expected from the work of others, thyroxine solutions of proper concentrations (above $8\frac{2}{3}$) induce metamorphosis; but in no case does one uniform concentration of thyroxine reproduce the normal metamorphic time-table. It is this aspect of the results with which we are concerned and which will be subjected to analysis.

The three experiments differ in only one important particular. The animals in experiments 2 and 3 were in general much more sensitive to thyroxine than those in experiment 1. For example, concentration $8\frac{2}{3}$ yielded about the same result in 2 and 3 as concentration 8 did in experiment 1. The reason for this difference is not apparent, although differences in temperature in the early days of the experiments may constitute a factor. My experience has convinced me that the sensitivity in different experiments cannot properly be compared until the possible role of slight environmental factors has been evaluated and scrupulously controlled. No conclusions regarding sensitivity may validly be drawn from ordinary laboratory experiments and consequently the present experiments are not considered as furnishing any basis for a discussion of this question.

In this paper we are not concerned with absolute values but rather with sequence of events and rate of change. In these respects the three experiments are in excellent agreement.

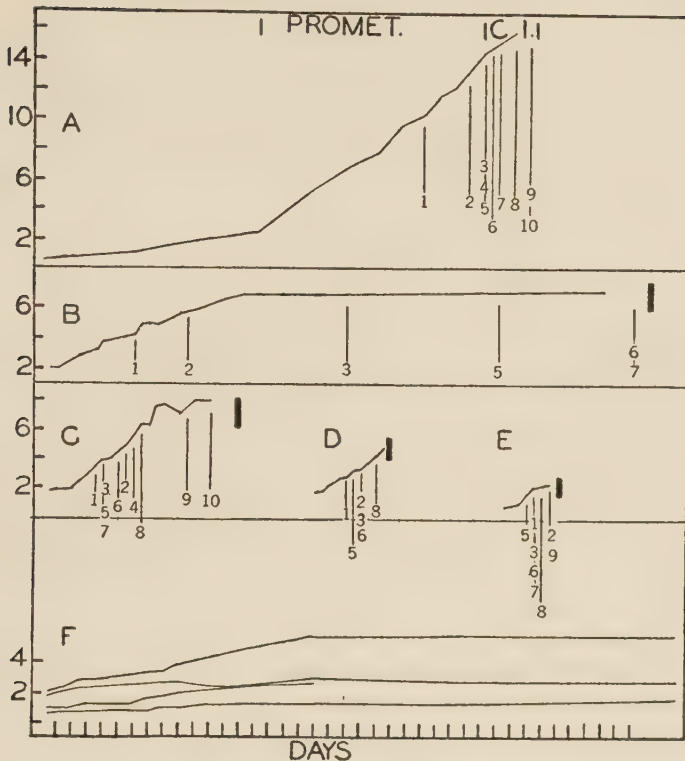


Fig. 1 The sequence of metamorphic events. A, in an animal metamorphosing normally. B, in an experimental animal at concentration $8\frac{1}{2}$. C, in concentration $7\frac{1}{2}$. D, in concentration 7. E, in concentration 6. F, operated controls in tap water. Each figure gives the curve for the growth of the hind legs. Metamorphic events are indicated by numbers according to the scheme given below. The line above the number marks the day and point upon the curve at which the event indicated was observed. A short vertical bar indicates the death of the animal. The significance of the numbers is as follows: 1, beginning of anal canal piece reduction, 2, resorption of anal canal piece completed, 3, first noted skin window for the fore leg formation or reduction of spiracular tube, 4, emergence of one or both fore legs, 5, lips markedly reduced, horny teeth lost, 6, one or both horny beaks lost, 7, first evidence of gross tail fin resorption, 8, tail fin completely resorbed, 9, tail reduced to a black knob, 10, mouth widening completed.

The abscissa is marked off for time; each indicated interval representing 1 day. The hind leg length in millimeters is plotted on the ordinates. In figure A the period of prometamorphosis is marked off from the shorter climax (CL.).

Because the thyroidectomies were successful in only part of the operated animals, some tadpoles in the experiments might be expected to metamorphose as a result of the activity of their own thyroids. This apparently happened in the case of four animals considered later, which underwent normal metamorphosis in dilute thyroxine concentrations in experiment 3.

4. The sequence of events in experimental animals

If we consider first the response of animals to dilute effective concentrations, such as $8\frac{1}{3}$, we notice that the sequence of events is very much like that of normal metamorphosis. Figure 1 B gives a graphic picture of the responses of one such animal, entirely representative of its group. First the hind legs begin to grow, then the anal canal piece is resorbed, the resorption of the spiracular tube follows, and mouth changes and tail regression are initiated. Figures 1 C and 1 D illustrate intermediate behavior at concentrations $7\frac{1}{3}$ and 7, respectively.

As the concentration of thyroxine increases, the sequence of events may be somewhat distorted by the earlier beginning of change that normally comes late. For example, at concentrations 7 or 6, tail regression begins before hind leg growth or resorption of the anal canal piece have made very much progress. This distortion of metamorphosis has been frequently noted and commented upon by older workers. It represents a crowding together of events that are well spaced in normal metamorphosis. It is evident, however, from the present experiments that the sequence of occurrences is inherent in the part responding to thyroxine and is independent of the concentration of thyroxine. Concentration may crowd the events together but does not alter their sequence. This can be made evident even with the strongest concentrations employed if we note the time of beginning of the various changes, such as the initiation of hind leg growth, the beginning of anal canal piece resorption, etc. The cause of the metamorphic sequence must therefore be sought in the tissues themselves.

5. *Time-table in induced metamorphosis*

It has just been pointed out that the more dilute concentrations give approximately normal sequences. Yet comparison with figure 1 A shows that in no case is a normal metamorphic picture obtained. The discrepancy between normal and thyroxine-induced metamorphosis lies in the time occupied by the various events. Under the conditions of the experiments, the time interval from the beginning of rapid hind leg growth (after the buds have reached about 3 mm. length) to the advanced resorption of the anal canal piece is normally 10 to 15 days. The complete regression of the tail then usually occupies an additional 4 days. In a concentration of $8\frac{1}{3}$, the first part of metamorphosis may be prolonged to occupy about the normal time. But then in such a concentration, the events of the climax are exceedingly widely separated and prolonged instead of following quickly upon one another. Observation of such animals gives the impression that metamorphic change has ceased, but graphic representation (fig. 1 B) reveals that it is merely extremely slowed.

At a concentration of about 7, the time intervals of the events of the metamorphic climax are approximately normal; but then the prometamorphic period is abnormally hastened, the climax changes appearing before the hind legs have had sufficient time to grow or the anal canal piece to be resorbed. Again at concentrations 6 or 5, the entire picture is distorted by the abrupt precipitation of all events almost simultaneously. No values can be found which will yield a normal time-table since any concentration is either too low to cause a sufficiently rapid succession of climax events or too high to allow sufficient time for prometamorphic changes. The best that can be achieved with intermediate concentrations ($7\frac{1}{3}$) is shown in figure 1 C. This analysis of the results with graded concentrations of thyroxine leads to the conclusion that although dilute solutions may induce the normal sequence of events, no single concentration reproduces the normal metamorphic time-table.

6. Relation between thyroxine concentration and rate of metamorphic change

From what has been said it is obvious that the rate of tissue activity in metamorphosis varies directly with the concentration of thyroxine. This general relationship has, of course, long been known. Here an attempt will be made to analyze this relation quantitatively. The available data for the more significant changes have been plotted from experiment 3 in figure 2 and examples from experiments 1 and 2 have also been included for comparison. It should be noted that in these curves the ordinal axis is a logarithmic scale and that the abscissa is likewise logarithmic since the exponent of the dilution is plotted thereon. This type of graph is more convenient for covering a wide range of data and further presents certain interpretational advantages.

These curves show that the change in time for a given change in concentration of thyroxine is remarkably alike for all of the events studied. With dilution there is at first a slow increase in time but soon further small diminutions in concentration greatly increase the time required so that the curve becomes exceedingly steep. The time required does not appear to be a linear function of concentration but a precise analysis of the form of these curves must be left for further study. For the stronger concentrations, each curve tends to flatten out to the same minimal time value necessary for the beginning of each separate event, the time being about $1\frac{1}{2}$ to 2 days in experiment 3. It is only in the more dilute concentrations that the time requirements for the several events are seen to be different. This is of course only another way of saying that all events tend to bunch together in high concentrations and to spread widely in low concentrations.

The curves in figure 2 further afford no evidence for the conception of an absolute threshold of concentration for an event but rather indicate that given time enough any concentration will induce all metamorphic events. Of course such a conclusion cannot be stated for concentrations or time intervals beyond those covered in the present study but within

the range investigated differential thresholds are not in evidence. The conclusion of Allen ('32) that the threshold concept can be applied only when the time of action is stated is thus substantiated, or as he puts it: "This 'threshold of response' is really a question of time of response."

Attention may be called to the fact that the curves given in figure 2 include all of the animals in experiment 3 (except the four which metamorphosed normally). They illustrate the degree of the consistency in the behavior of different

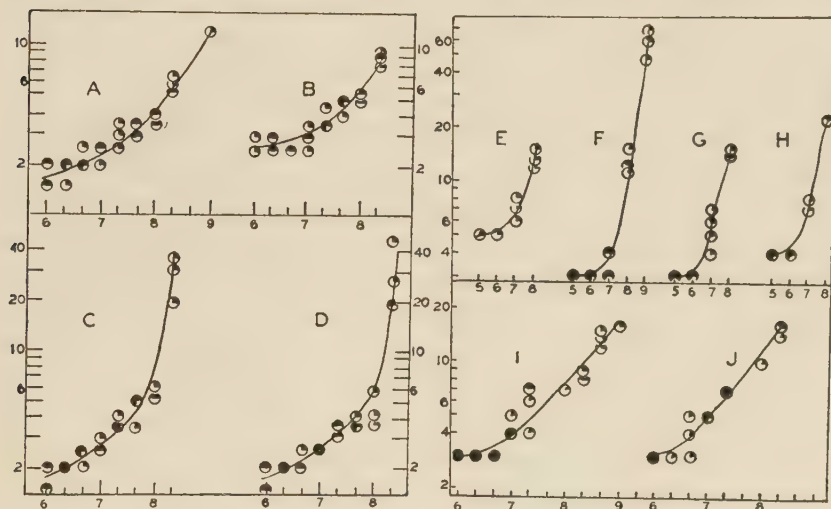


Fig. 2 The relation between thyroxine concentration and the time of metamorphic events. The abscissae are marked off for the concentrations which are designated by the scheme adopted in the text. The ordinates indicate time in days, a logarithmic plot being employed. A) Time required till the first appearance of resorption in the anal canal piece. Experiment 3. B) Time till the completion of anal canal piece resorption. Experiment 3. C) Time till resorption of lips is complete. Experiment 3. D) Time till tail fin shows first evidence of resorption. Experiment 3. E) Time till anal canal piece is completely resorbed. Experiment 1. F) Time till lips are resorbed. Experiment 1. G) Time till first noted tail fin resorption. Experiment 1. H) Time till tail fin is completely resorbed. Experiment 1. I) Time till anal canal piece resorption is complete. Experiment 2. J) Time till tail fin resorption is complete. Experiment 2.

To indicate the number of cases at each locus the circles are filled in as follows. In curves A to D inclusive, each quarter of the circle filled in represents one individual. In E to H inclusive, each eighth of the circle represents one individual and in I and J each fifth stands for one animal.

animals and the validity of using single cases to illustrate general relations.

7. Influence of size

It was originally intended to study this problem by comparing the sensitivity of animals of different ages to the same thyroxine concentration. The three experiments were arranged with this end in mind. However, it soon became apparent that unknown and uncontrolled environmental factors introduced so much variation that the responses in different experiments could not validly be compared. Therefore two groups of tadpoles which had reached full size or over as a result of special care were run simultaneously with the smaller animals in experiment 3 at concentrations $7\frac{1}{3}$ and 8. The results gave little difference between the two size groups, but such differences as appeared indicate a slower response on the part of the larger animals. For example, the smaller tadpoles required as averages 4.6 and 4.5 days for events which occurred in the full grown group in 4.7 and 5.1 days. That the sensitivity of the tissues to thyroxine is not altered in the later tadpole stages is further evidenced by the fact that throughout the experiments no differences were noted between the larger and smaller animals of any group.

In evaluating this conclusion, it must, however, be borne in mind that the small number of animals used would permit the detection of only gross changes in sensitivity and further that the animals compared were of the same age, differing only in size. Size, however, is known to affect the time of metamorphosis (Adolph, '31). It appears from my results that this size factor in time of metamorphosis does not operate through any marked increase in susceptibility of the tissues to thyroxine.

8. The influence of the thyroid gland on the characteristic reactions to thyroxine

As already remarked it is practically certain that in each experiment some of the animals used were thyroidectomized

and others not. Nevertheless, in experiments 1 and 2, where the animals were less than half-grown at the start, their response to thyroxine was highly uniform. This suggests that the presence or absence of the thyroid gland is unimportant for the reaction to thyroxine. This conclusion is further supported by a comparison of the unoperated control with

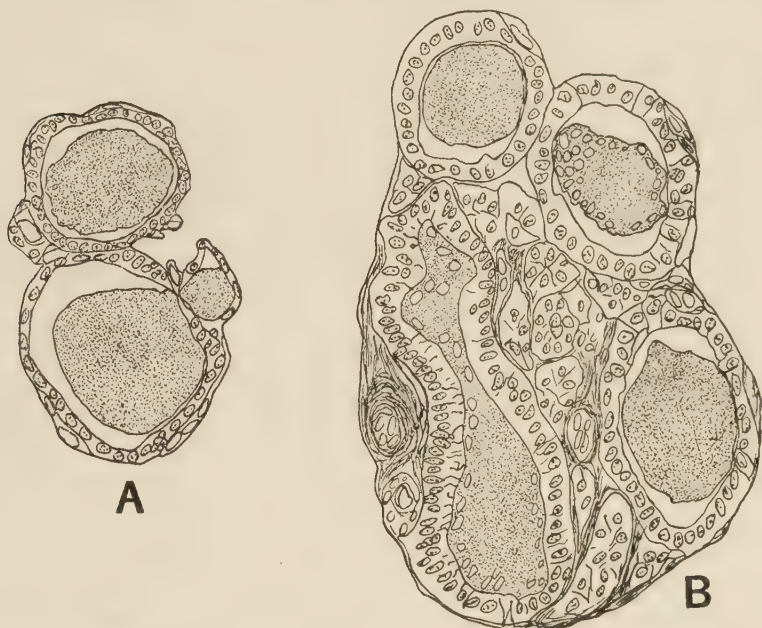


Fig. 3 Camera lucida drawings of sections through two thyroid glands. A, is from an animal kept in concentration 9 and showing the reactions described as characteristic. B, is from an animal kept at concentration $9\frac{1}{2}$, but which showed a normal metamorphic picture instead of the slow reaction expected. Both figures at the same magnification. $\times 150$.

the operated experimental animals in experiment 2. These groups ran almost parallel throughout the experiment.

In experiment 3, where larger animals were employed, the results for the most part support the foregoing conclusion, with the exception of the four animals already mentioned. Whereas the other animals in this experiment exhibited in the more dilute concentrations a protracted metamorphosis

or an absence of metamorphic events beyond hind leg growth, these four animals, in sharp contrast to their fellows, underwent normal metamorphosis. They were killed near the end of metamorphosis and examination of sections revealed in all four the presence of large thyroid glands, which closely resemble those of normal animals undergoing metamorphosis. Twenty animals which gave characteristic responses to the thyroxine solutions have also been examined histologically; in eleven cases no trace of thyroid tissue was found and in the other nine the glands were small and generally had a low epithelium. Representative sections are given in figure 4 and show the sharp difference between the thyroid of the four normally metamorphosing animals and the small glands of animals metamorphosing in response to thyroxine. The latter vary somewhat among themselves but do not approach the appearance of the former.

These facts seem clearly to warrant the conclusion that in the characteristic reaction of young tadpoles to thyroxine solution, their own thyroid glands play little if any part. But if the exposure to thyroxin begins after the animal's own thyroid has entered upon its characteristic growth phase in prometamorphosis, then the animal may undergo a normal transformation as a result of the activity of its own thyroid. This seems to have occurred in the four cases mentioned above which are therefore excluded from the discussions.

It may be concluded that the presence of a thyroid gland in its premetamorphic state is largely immaterial to the reactions described. The thyroxine solutions do not act by influencing the thyroid gland.

AN ATTEMPT TO REPRODUCE THE NORMAL METAMORPHIC PICTURE

From what has been said it is evident that, although a single concentration of thyroxine does not induce normal metamorphosis, this might be accomplished by a proper manipulation of concentrations. By starting with low concentrations which incite approximately normal early metamorphic events and then changing gradually to higher

concentrations, a more or less normal sequence and spacing of climax results should be attained. This idea was experimentally tested as follows (experiment 4).

Ten animals which had already passed the size and time at which the great majority of normal control animals of the batch had metamorphosed were selected from clutch 7. These

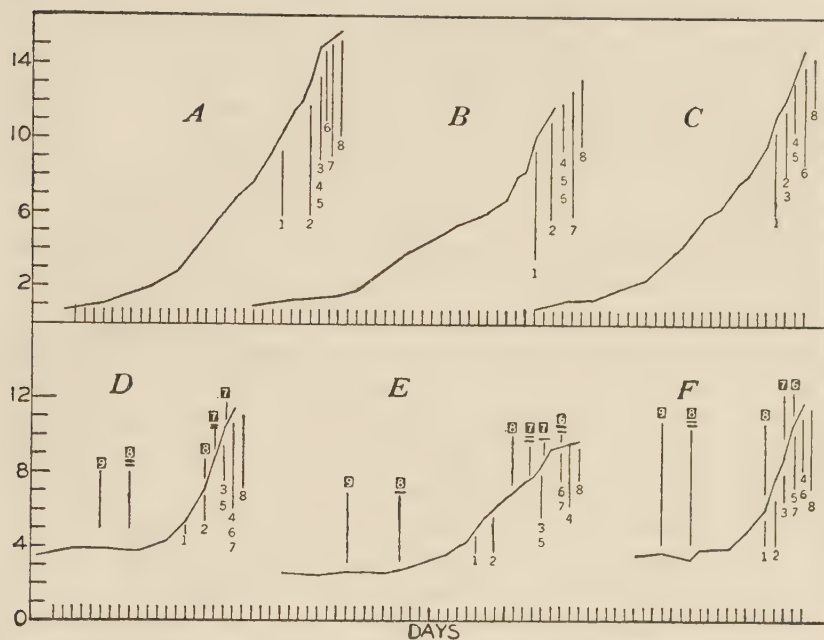


Fig. 4 Curves showing some results in the attempt to duplicate the normal metamorphic pattern. Figures A, B and C give examples of normal metamorphosis, which served as models for the treatment of the experimental animals, D, E and F. The arrangement of the curves is as in figure 1. In addition the white numbers on the black background indicate the concentrations employed at various times. A single bar below such a number indicates that the concentration used is one-third higher than the number, two bars indicate two-thirds. Each abscissa marker denotes 1 day. The ordinates give hind leg length in millimeters.

were animals which presumably had been successfully thyroidectomized. They were kept under observation for 1 week in tap water to determine the rate of hind leg growth. They were then introduced into concentration 9 and watched daily. When progress seemed suitable in each case, the concentration was raised, the exact procedure being adjusted to the

response shown by each animal. Unfortunately but little more than a month's time was available for this experiment and consequently the time during which the animals could be allowed to remain in the dilute concentrations was limited.

The results in the more successful cases were very much alike. In figure 4 are presented the curves of three of the experimental animals D, E and F and three curves, A, B, C, of normally metamorphosing animals, which served as models of what was to be aimed at. The concentrations used to induce metamorphosis are given above the base of the curves D, E and F. It is seen that during the week in tap water there was practically no hind leg growth or other metamorphic change. About a week after the application of low concentrations, hind leg growth began and was maintained at approximately the normal rate until the experiment was terminated by the death of the animals. The sequence of other morphological changes is approximately normal in the three cases, but the hind leg length at which these events occur is somewhat smaller than normal. In short, not enough time was allowed for the growth of the hind legs before later metamorphic events were precipitated. This difference from the normal could probably be corrected by longer exposure to dilute concentrations. Although, therefore, the results were not entirely satisfactory, comparison with the curves in figure 1 shows that to a large extent the objects of the experiment have been accomplished and that a picture for the second half of prometamorphosis through the climax which closely approximates the normal has been obtained.

THE EFFECT OF TEMPORARY IMMERSION IN THYROXINE SOLUTIONS

For the critical evaluation of the foregoing experiments, it is desirable to know something of the relation between the external and internal concentration of thyroxine. It is natural to assume that the latter is some function of the former and this assumption is general with previous workers. It is, however, possible that the animal actively extracts thy-

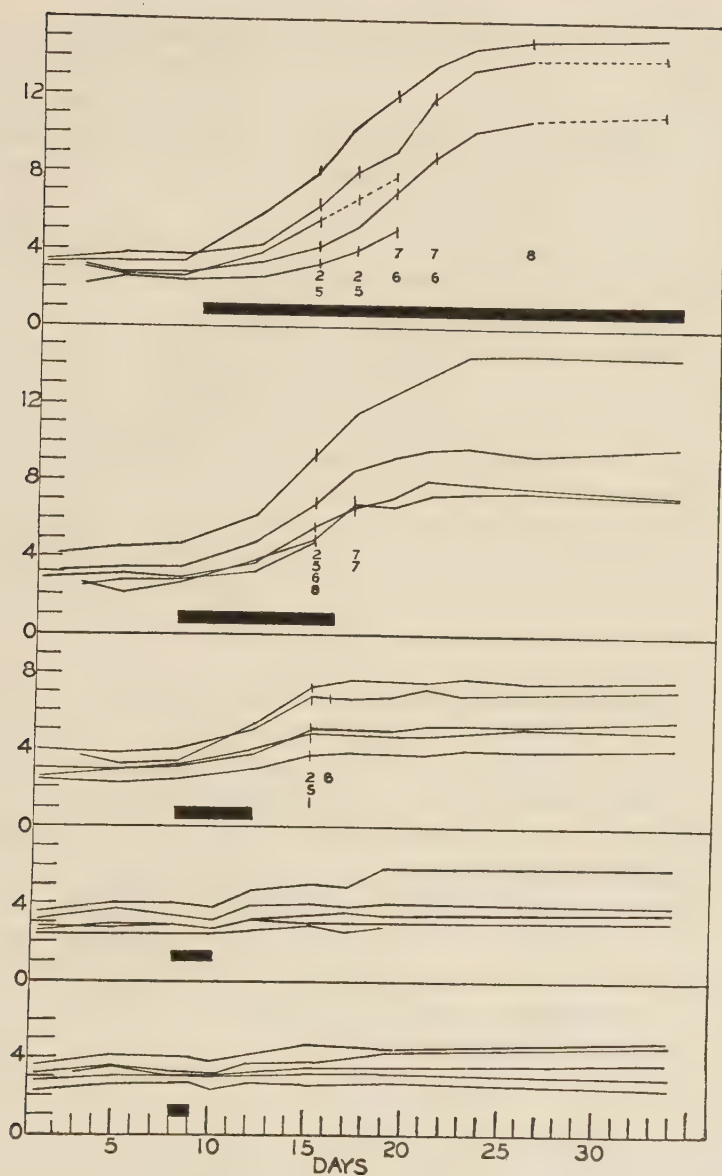


Fig. 5 Curves giving the results of an experiment on temporary immersion of thyroidectomized tadpoles in thyroxine concentration of 8. The arrangement is as in figure 2. The abscissa markers indicate 1 day each. The hind leg length is indicated in millimeters on the ordinates. The black bar in each group indicates the period of time during which the tadpoles were subjected to thyroxine.

roxine from the medium and builds up the internal concentration until this passes the threshold values for the various metamorphic events. Various general arguments might be arrayed against this theory, such as the relatively prompt excretion of hormones in mammals, but an experimental test seemed desirable (experiment 5).

Twenty-five fully grown operated tadpoles, like those used in the preceding experiment, were divided into five groups of five each. After a preliminary week in tap water, they were subjected to concentration 8. One, 2, 4 and 8 days later a group of five was removed to tap water, one group being left in thyroxine throughout the experiment. The results are again best presented in graphic form, figure 5. It is seen that all of the animals react in much the same way while in the thyroxine solution, but within a few days after removal to tap water the metamorphic changes which were initiated cease to progress further. The hind leg growth and tail regression come practically to a halt and no new metamorphic events appear.

It is clear, therefore, that the continuous presence of thyroxine in the environment is essential for the maintenance of metamorphic changes. The thyroxine absorbed is apparently quickly lost and does not accumulate into higher concentration than present in the medium. Consequently explanations based on the idea of internal thyroxine accumulation until tissue thresholds are reached do not seem very probable. Although in my opinion, the problem is still obscure, the evidence favors the view that internal concentration is in equilibrium with and a function of external concentration of thyroxine.

DISCUSSION

This discussion will concern only those aspects of the present report which seem to me to throw light upon the problem of the relation between the activity of the thyroid gland and the pattern of metamorphic events. The hypothesis to be developed, although consistent with the experimentally de-

terminated facts, is largely theoretical and useful only as suggesting further analyses. I will assume throughout that thyroxine acts essentially like the thyroid hormone and that its concentration in the tadpole blood is a direct function of that in the surrounding medium under the conditions of the foregoing experiments.

It might first be pointed out that the characteristic pattern of metamorphic events may be analyzed into two factors: the sequence of events, and the time relationships between successive events. The sequence of events appears from the foregoing analysis to be inherent in the tissues concerned rather than controlled by the activating factor. The time intervals between events, on the other hand, are clearly dependent upon the concentration of the activating substance. For the attainment of the normal time-table, the thyroid gland must have something like the following ontogenetic history.

1. Premetamorphosis

The results of the attempts to reproduce the normal metamorphic picture indicate that a considerable period of stimulation by a very low concentration of thyroxine is necessary before a slightly higher concentration will induce normal prometamorphic hind leg growth. It is therefore suggested that before metamorphosis, the thyroid gland is slightly active and prepares the hind legs for a long period of rapid growth when the concentration of thyroxine is later raised slightly.

2. Initiation of prometamorphosis

Before prometamorphosis, the growth of the hind legs is about proportionate to that of the body. The rapid prometamorphic growth of the hind legs must be caused by a rise in the activity of the thyroid gland. The amount and rate of rise must be such as to allow of almost maximal rate of hind leg growth, yet not sufficient, at first, to precipitate climax phenomena. It is thus conceived that the induction of

prometamorphosis results not from a change in the sensitivity of the tissues (see p. 330) but to a change in the activity of the thyroid gland.

3. Continuation of prometamorphosis

To induce normal metamorphosis the concentration of the hormone must be sufficient to produce the later events at their maximal rate. As we have seen this is only possible by the presence of a higher concentration than was allowable at the beginning of hind leg growth. If it is correct that thyroxine is rapidly lost from the body, this increased concentration could not result from the accumulation in the body of slowly secreted hormone, but must arise through more rapid elaboration and release of hormone by the thyroid gland. An increasingly active thyroid gland must then be postulated as characteristic of prometamorphosis.

4. Climax phenomena

A relatively high concentration of thyroxine is necessary for the production of a normal climax picture. Great activity of the gland at this time must therefore be predicated.

It may be remarked that the growth curve of the thyroid as revealed in the morphological studies of Morita ('32), Etkin ('30), and Allen ('19) is precisely in accord with the requirements of the foregoing theory. Further discussion will be omitted as this matter is at present under investigation.

The significant feature of the interpretation offered is that it explains a specific developmental time pattern by a purely quantitative mechanism, a variation in hormone concentration.

SUMMARY

1. The possible role of thyroxine concentration in determining the normal pattern of events in anuran metamorphosis was studied experimentally. *Rana cantibrigensis* tadpoles were thyroidectomized and when half to fully grown were subjected to various thyroxine concentrations. The sequence of metamorphic events was then observed in living animals, isolated singly.

2. The sequence of metamorphic events is independent of the concentration used, being essentially normal in all concentrations. This sequence appears to be inherent in the parts or organs concerned.

3. The rate of response is a function of the concentration. As the concentration is decreased, the time required for a given event at first rises slowly, then more abruptly.

4. Neither the size of the animal nor the presence or absence of the thyroid gland in later tadpole stages plays any significant role in the nature of the response.

5. The normal time spacing of the events cannot be attained by any single concentration of thyroxine. The more dilute effective concentrations reproduce the early metamorphic events with practically normal time intervals, but the later events are greatly slowed. The stronger concentrations may give a normal time spacing of the later climax events, but the early prometamorphic events are abnormally hastened.

6. An approximately normal time spacing of the metamorphic events can be obtained by gradually increasing the concentration of thyroxine as metamorphosis progresses.

7. It is suggested that in normal metamorphosis the thyroid gland becomes increasingly active. This theory is supported by studies on the development of the thyroid gland of *Anura*.

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